

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/15534 A1

- (51) International Patent Classification?: A01N 61/00, 61/02, 31/00 [AU/AU]; RMB 145 Wisemans Ferry Road, Maroota, NSW 2756 (AU).
- (21) International Application Number: PCT/AU00/01018 (74) Agent: F B RICE & CO; 605 Darling Street, Balmain, NSW 2041 (AU).
- (22) International Filing Date: 28 August 2000 (28.08.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: PQ 2469 26 August 1999 (26.08.1999) AU
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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
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- Published:
— With international search report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/15534 A1

(54) Title: INSECTICIDAL COMPOSITION

(57) Abstract: An insecticidal composition is disclosed that includes *Tasmannia stipitata* extract in combination with an insecticidally effective oil such as petroleum oil, vegetable oil, and fish oil. The *Tasmannia stipitata* extract enhances the insecticidal activity of these oils. It is particularly useful against a variety of insect pests that occur on plants such as thrips, aphids, mites, scales and caterpillars. It is also useful against lice and flies.

INSECTICIDAL COMPOSITION

Technical Field

The present invention relates to an insecticidal composition including *Tasmannia stipitata* extract and insecticidally effective oils such as petroleum oils, vegetable oils and/or fish oils. The invention also relates to a method of controlling arthropods pests using combinations of *Tasmannia stipitata* extract and insecticidally effective oils such as petroleum oils, vegetable oils and/or fish oils.

Background Art

The genus *Tasmannia* was formerly known as *Drimys*. *Tasmannia stipitata* (also known previously as *Tasmannia aromatica* var. *pendunculata* or *Drimys aromatica*) is commonly known as Dorrigo Pepper. It is rich in the compound polygodial.

Polygodial is used in foods for its hot and spicy taste. It also has been found to have antifungal, antibacterial and insecticidal activity.

The active ingredients of *Tasmannia stipitata* appear to be contained within oil cells concentrated in new leaves, new stems and berries. Extracts of leave, stems and berries include polygodial as well as linalool, pinene, copaene, caryophyllene and bicyclo-germacrene.

Petroleum oils (or mineral oils) are used as insecticides. Certain vegetable oils also have insecticidal activity. The most common is canola oil (rapeseed oil). Sesame oil is also used. Other vegetable oils having insecticidal activity include mustard oil. A third group of oils having insecticidal activity is fish oil.

Disclosure of Invention

Combinations of *Tasmannia stipitata* extract and petroleum oil or insecticidally effective vegetable oil have been found to have unexpected synergistic activity as insecticides. It is assumed that the insecticidal activity of fish oils would be similarly enhanced in combination with *Tasmannia stipitata* extract.

Accordingly, the present invention provides an insecticidal or insect repellant composition including *Tasmannia stipitata* extract and an insecticidally effective oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one fish oil and mixtures thereof.

In addition to insecticidal activity, the composition acts as an insect repellent. It has also been found that insects that come into contact with a surface that has been treated with the composition exhibit reduced feeding and oviposition (egg laying) behaviour. The composition may therefore be used to control insects.

In another aspect, the present invention provides a method of controlling or killing insects comprising exposing an insect population to a composition including *Tasmannia stipitata* extract and an insecticidally effective oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one fish oil and mixtures thereof.

In a further aspect, the present invention provides for the use of *Tasmannia stipitata* extract to enhance the insecticidal activity of an oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one fish oil and mixtures thereof.

The extract of *Tasmannia stipitata* is preferably prepared by solvent extraction of new leaves, new stems and berries. The concentration of polygodial in the extract will vary with the relative proportions of leaves, stems and berries extracted and will also vary with season. Concentrations of about 10% or more polygodial are desirable in the extract, preferably about 10-40% and more preferably about 25-38%.

The concentration of *Tasmannia stipitata* extract in the insecticidal composition may range from about 0.01% w/v to about 1.25% w/v, preferably 0.02% w/v to 1.0% w/v, more preferably 0.03% w/v to 0.125% w/v. The desired concentration will vary with the polygodial content of the *Tasmannia stipitata* extract.

The concentration of insecticidally effective oil in the insecticidal composition may range from about 0.25% w/v to about 1.5% w/v, preferably 0.5% to 1.0% w/v.

Although it will be appreciated that the composition of the invention may be applied to insect populations in a variety of ways, spraying is one preferred means. Application of a spray may be achieved using aerosols, suitably pressurised dispensers or other pumping devices.

Insects of course reside in a variety of environments. For those environments that are animate, such as in the case of plants and animals that are infested with insects, the present invention is advantageous in that it has

reduced toxicity. This arises out of the fact that due to enhanced insecticidal activity, less active material need be applied to the plant or animal.

Modes for Carrying Out the Invention

Materials

- 5 1. Samples of *Tasmannia stipitata* extract (hereinafter referred to as "stipitata extract") were prepared by solvent extraction of mixtures of new leaves, new stems and berries of *Tasmannia stipitata*. These samples contained an average of 27.6% polygodial. The polygodial content of the samples was ascertained as follows:
- 10 A known weight of stipitata extract was dissolved in 5 mL of ethanol with an internal standard of tridecane at a concentration of 2mg/mL. This solution was analysed by gas chromatography and the polygodial was assessed by area ratio to the tridecane and assumption that the response factor between the two compounds equals 1. The average percent of
- 15 polygodial stated above is the average percent of volatiles in the extract.
2. D-C-Tron Plus®, spray oil, supplied by Ampol, active ingredient 839g/L petroleum oil (referred to in the tables as "Petroleum Oil 2").
3. Fasta® oil, supplied by Cobbett Pty Ltd, 704 g/L vegetable oil, primarily canola oil (hereinafter referred to as "canola oil").
- 20 4. White oil spray, supplied by Yates, active ingredient 820g/L petroleum oil (hereinafter referred to as "white oil" and in the tables as "Petroleum Oil 1").

Process

- 25 Stipitata extract in amounts shown in column A of the table below was combined with oils B, C or D respectively in amounts as shown below.

	A	B	C	D
Mixtures	Weight of Stipitata extract (g)	Weight of D-C-Tron Plus® (g)	Weight of Canola Oil (g)	Weight of white oil (g)
1	1.25	10	10	10
2	0.625	10	10	10
3	0.3125	10	10	10
4	1.25	5	5	5

Each of these blends was diluted with distilled water in a 100ml volumetric flask to prepare the following mixtures: Stipitata extract(ml) and oil(ml) (1.25 and 10), (0.625 and 10), (0.3125 and 10) and (1.25 and 5) per 100ml dilution.

From these homogenised stock solutions, further serial dilutions to (0.125 and 1), (0.0625 and 1), (0.03125 and 1), (0.125 and 0.5), (0.0625 and 0.5) and (0.03125 and 0.5) per cent were prepared by mixing the required amount of the stock solution in distilled water.

For Stipitata extract alone, one gram was dissolved in 20ml of absolute ethanol and distilled water was added to prepare a 1% stock solution. A drop weighing 40mg Triton X-100® surfactant supplied by Union Carbide Chemicals and Plastic Co, was added to the stock solution. Further serial dilutions of 0.01, 0.02, 0.04, 0.06 and 0.08% were prepared by mixing the required amount of stock solution with distilled water.

For each oil alone a 10% w/v emulsion in distilled water and further dilutions of 2.0, 1.8, 1.6, 1.4, 1.2 1.0, 0.50 and 0.25% were prepared by adding to the required amount of distilled water.

Adult female two-spotted mite (*Tetranychus urticae*) were collected. Tests were conducted on 80 females for each treatment, distributed on four French bean leaf discs 25mm diameter, contained in 90mm diameter Petrie dishes. Each treatment was repeated twice.

The leaf discs were placed on moist cotton wool covered with muslin netting.

Water was added to the dish daily to prevent the desiccation of the leaf discs.

The dose of product for testing was applied by means of a Potter Precision Spray Tower and a 5ml aliquot was used for each Petrie dish.

The mortality (number killed) was assessed 24, 48 and 72 hours after treatment. Death was recognised by the absence of movement when the test mites were mechanically stimulated.

Probit analysis based on the concentration of Stipitata extract in the mixtures was carried out to interpret the dose mortality relationship.

Results

Results are summarised in the tables below.

Product	Spray oil conc %	Time in Hours	LD ₀₅ and 95% Confidence Limits		
			Lower	Median	Higher
Stipitata		24	0.13	0.19	0.3
		48	0.06	0.12 (a)	0.77
Petroleum Oil 2		24	1.75	2.72 (c)	67.06
Petroleum Oil 1		24	2.50	3.04	4.23
		48			
Canola		24	3.85	4.60	5.61
		48	3.41	3.71 (g)	4.47
Petroleum Oil 2 + Stipitata	0.5	24		0.196	
	1.0	24		0.07	
	0.5 (d)	48	0.006	0.048 (b)	0.085
	1.0	48	0.03	0.04	0.06
	0.5	72		0.063	
	1.0	72		0.03	
Canola oil + Stipitata	0.5	24		0.12	
	1.0	24	0.037	0.068	0.325
	0.5	48		0.11	
	1.0 (f)	48		0.08 (e)	
	0.5	72		0.069	
	1.0	72		0.049	

Product	Spray oil conc %	Time in Hours	LD ₅₀ and 95% Confidence Limits		
			Lower	Medium	Higher
Stipitata		24	0.03	0.03	0.04
		48	0.02	0.03 (t)	0.03
Petroleum Oil 2		24	0.44	1.23 (w)	12.85
Petroleum Oil 1		24	1.23	1.54	1.88
		48			
Canola		24	1.81	2.02	2.34
		48	1.54	1.69 (x)	1.88
Petroleum Oil 2 + Stipitata	0.5	24		0.023	
	1.0	24		0.02	
	0.5 (v)	48	0.001	0.006 (u)	0.011
	1.0	48	0.002	0.01	0.017
	0.5	72		0.016	
	1.0	72		0.01	
Canola + Stipitata	0.5	24		0.03	
	1.0	24		0.01	
	0.5	48		0.02	
	1.0 (z)	48		0.017 (y)	
	0.5	72		0.018	
	1.0	72			0.017

Combined with all the oils outlined above, Stipitata extract was more efficacious (ie. caused higher mortality) than when used alone. In addition, for oils to which Stipitata extract had been added, efficacy was significantly enhanced. For oils used alone, mortality was lowest of all treatments.

5 The results above demonstrate synergistic insecticidal effect against Two-spotted mite by *Tasmannia stipitata* extract mixed with different types of oils. The LD₀₅ of Stipitata extract alone is improved from 0.12% (a) to 0.048% (b) when blended with 0.5% petroleum oil. Simultaneously the LD₀₅ of petroleum oil is improved from 2.72% (c) to 0.5% (d) with the addition of
10 Stipitata extract.

 The LD₀₅ of Stipitata extract alone is improved from 0.12% (a) to 0.08% (e) when blended with 1% canola oil (f). Simultaneously the LD₀₅ of canola oil is improved from 3.71% (g) to 1% (f) with the addition of Stipitata extract.

 The LD₅₀ of Stipitata extract alone is improved from 0.03% (t) to
15 0.006% (u) when blended with 0.5% petroleum oil (v). The LD₅₀ of petroleum oil is simultaneously improved from 1.23% (w) to 0.5% (v) with the addition of Stipitata extract. The LD₅₀ of Stipitata extract is improved from 0.03% (t) to 0.017% (y) when blended with 1% canola oil (z). The LD₅₀ of canola oil is simultaneously improved from 1.69% (x) to 1% (z) with the
20 addition of Stipitata extract

 Synergistic activity as an insecticide against a broad range of insects is expected including thrips, aphids, scales, mites, caterpillars, lice and flies.

Industrial Applicability

 Combining *Tasmannia stipitata* extract with insecticidally effective oils
25 including petroleum oil, vegetable oil, and fish oil gives a highly effective insecticidal composition. The proportion of active ingredients in the composition required for effective control of the insect pest can be substantially reduced compared to the proportion required for each ingredient when used alone. Phytotoxicity of the composition is significantly
30 reduced by reduced actives levels, as is the cost of manufacturing the composition. It is expected that the insecticidal composition will be effective against a broad range of insects.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to
5 be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. An insecticidal or insect repellant composition including *Tasmannia stipitata* extract and an insecticidally effective oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one
5 fish oil and mixtures thereof.
2. An insecticidal or insect repellant composition as claimed in claim 1 wherein the *Tasmannia stipitata* extract is included in the composition in an amount of from about 0.01% to about 1.25% w/v, preferably 0.02% to 1.0%
10 w/v, most preferably 0.03% to 0.125% w/v.
3. An insecticidal or insect repellant composition as claimed in claim 1 or claim 2 wherein the insecticidally effective oil is included in the composition in an amount of from about 0.25% to about 1.5% w/v, preferably 0.5% to
15 1.0% w/v.
4. An insecticidal or insect repellant composition as claimed in any one of claim 1 to 3 wherein the insecticidally effective oil is petroleum oil.
- 20 5. An insecticidal or insect repellant composition as claimed in any one of claims 1 to 3 wherein the insecticidally effective oil is vegetable oil.
6. An insecticidal or insect repellant composition as claimed in any one of claims 1 to 5 wherein the composition is an emulsion with water.
25
7. A method of controlling or killing insects comprising exposing an insect population to a composition including *Tasmannia stipitata* extract and an insecticidally effective oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one fish oil and mixtures
30 thereof.
8. A method as in claim 7 wherein the composition is applied to the insect population by spraying.
- 35 9. A method as in claim 7 or claim 8 wherein the insect population is resident on a plant or an animal.

10. A method as in claim 9 wherein the insect population is resident on a plant.
- 5 11. A method as in claimed in any one of claims 7 to 10 wherein *Tasmannia stipitata* extract is included in the composition in an amount of from about 0.01% to about 1.25% w/v, preferably 0.02% to 1.0% w/v, most preferably 0.03% to 0.125% w/v.
- 10 12. A method as claimed in any one of claim 7 to 11 wherein the insecticidally effective oil is included in the composition in an amount of from about 0.25% to about 1.5% w/v, preferably 0.5% to 1.0% w/v.
13. A method as claimed in any one of claim 7 to 12 wherein the
15 insecticidally effective oil is petroleum oil.
14. A method as claimed in any one of claims 7 to 12 wherein the insecticidally effective oil is vegetable oil.
- 20 15. A method as claimed in any one of claims 7 to 14 wherein the composition is an emulsion with water.
16. Use of *Tasmannia stipitata* extract to enhance the insecticidal activity of an oil selected from the group consisting of at least one petroleum oil, at
25 least one vegetable oil, at least one fish oil and mixtures thereof.
17. The use of claim 16 wherein *Tasmannia stipitata* extract is included in the composition in an amount of from about 0.01% to about 1.25% w/v, preferably 0.02% to 1.0% w/v, most preferably 0.03% to 0.125% w/v.
30
18. The use of claim 16 or claim 17 wherein the insecticidally effective oil is included in the composition in an amount of from about 0.25% to about 1.5% w/v, preferably 0.5% to 1.0% w/v.
- 35 19. The use as claimed in any one of claim 16 to 18 wherein the insecticidally effective oil is petroleum oil.

20. The use as claimed in any one of claims 16 to 18 wherein the insecticidally effective oil is vegetable oil.

5 21. The use as claimed in any one of claims 16 to 20 wherein the composition is an emulsion with water.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01018

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. ⁷: A01N 61/00, 61/02, 31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: Chem Abs (CA), HCA, Agricola, WPIDS

- (insecticid? or insect?(4a)repell?), (mite# or thrip# or aphid# or flies or fly), antifeedant#, (stipitata or drimys or tasmania(w)aromatica or dorrign(w)pepper), (polygodial#)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Powell, G., Hardie, J. and Pickett, J.A., 1995. Behavioural evidence for detection of the repellent polygodial by aphid antennal tip sensilla, <i>Physiological Entomology</i> , 20: 141-146 - see whole document	1-14
A	Asakawa, Y. <i>et al</i> , 1988. Activity of drimane antifeedants and related compounds against aphids, and comparative biological effects and chemical reactivity of (-)- and (+)-polygodial, <i>Journal of Chemical Ecology</i> , 14(10): 1845-55 - see whole document	1-14

☒ Further documents are listed in the continuation of Box C ☐ See patent family annex

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Date of the actual completion of the international search

19 September 2000

Date of mailing of the international search report

26 SEP 2000

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU00/01018

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Messchendorp, L., Gols, G.J.Z., and van Loon, J.J., 1998. Behavioural effects and sensory detection of drimane deterrents in <i>Myzus persicae</i> and <i>Aphis gossypii</i> nymphs, <i>Journal of Chemical Ecology</i> , 24(9): 1433-46 - see whole document	1-14